

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-115. **(Canceled)**

116. **(Currently amended)** A system for control of gene expression comprising:

(i) ~~[[a]]~~an isolated first nucleic acid molecule comprising a cis-repressive sequence element upstream of an open reading frame (ORF), or including part of the open reading frame, wherein the cis-repressive sequence element ~~first nucleic acid molecule~~ forms part of a stem-loop structure that represses translation of the ORF; and

(ii) ~~[[a]]~~an isolated second nucleic acid molecule comprising first and second stem-forming portions and a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion to form a loop, and wherein a portion of at least 6 nucleotides in length of the first or second stem-forming portion of the second nucleic acid molecule is complementary or substantially complementary to a portion of at least 6 nucleotides in length of the first nucleic acid molecule and interacts with the first nucleic acid molecule to disrupt the stem-loop structure in which the cis-repressive sequence element participates and thereby derepress translation of the ORF.

117-176. **(Canceled)**

177. **(Withdrawn)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

(i) a template for transcription of a cognate trans-activating RNA element;

and

(ii) a promoter located upstream of the template for transcription of the trans-activating RNA element; and

(c) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

178. **(Withdrawn)** A kit for allowing a user to regulate expression of a gene of choice comprising:

a plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

179. **(Withdrawn)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

- (i) a template for transcription of a cis-repressive RNA element; and
- (ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;
- (b) a second plasmid comprising
 - (i) a template for transcription of a cognate trans-activating RNA element; and
 - (ii) a promoter located upstream of the template for transcription of the trans-activating RNA element;
- (c) a third plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and
- (d) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

180. **(Previously presented)** A kit comprising:

one or more oligonucleotides comprising a crRNA sequence, one or more oligonucleotides comprising a taRNA sequence, or one or more oligonucleotides comprising a crRNA sequence and one or more oligonucleotides comprising a taRNA sequence, wherein the kit further comprises one or more items selected from the group consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control

plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

181. **(Withdrawn)** A method of regulating translation of an open reading frame comprising steps of:

introducing an engineered template for transcription of an mRNA into a cell and allowing mRNA transcription to occur resulting in a transcribed mRNA, wherein the template is engineered so that the transcribed mRNA comprises first and second nucleic acid elements that form a stem-loop structure that represses translation of the mRNA; and providing an engineered nucleic acid molecule that interacts with the mRNA so as to derepress translation of the mRNA to the cell.

182. **(Withdrawn)** The method of claim 181, wherein the engineered template comprises:

(i) a first stem-forming portion;
(ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary;
(iii) a non-stem-forming portion connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion; and
(iv) an open reading frame (ORF),

wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation of the ORF.

- 183-242. **(Canceled)**

243. **(Withdrawn)** The method of claim 181, wherein the engineered nucleic acid molecule comprises:

(i) a first stem-forming portion;
(ii) a second stem-forming portion; and
(iii) a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem forming portion and the 5' end of the second stem-forming portion to form a loop,

and wherein a portion of the nucleic acid molecule is complementary or substantially complementary, to a portion of the transcribed mRNA.

- 244. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 80%.
- 245. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 90%.
- 246. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 98%.
- 247. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 5 fold.
- 248. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 10 fold.
- 249. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 19 fold.
- 250. **(Previously presented)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of RNA.
- 251. **(Withdrawn)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA.
- 252. **(Withdrawn)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA and RNA.
- 253. **(Previously presented)** The system of claim 116, wherein the cis-repressive sequence element is positioned upstream of the ORF.

254. **(Currently amended)** The system of claim 116, wherein the first nucleic acid molecule comprises:
- (i) a first stem-forming portion;
 - (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary and at least 4 nucleotides in length, and
 - (iii) a non-stem-forming portion that forms a loop connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion, wherein the ~~engineered~~ two stem-forming portions and the non-stem forming portion of the first nucleic acid molecule ~~forms a~~ form the stem-loop structure that represses translation of the ORF ~~when positioned upstream of an open reading frame (ORF).~~
255. **(Previously presented)** The system of claim 254, wherein the first and second stem-forming portions of the first nucleic acid molecule are substantially complementary.
256. **(Currently amended)** The system of claim 116, wherein ~~at least~~ a portion of the cis-repressive sequence element of at least 4 nucleotides in length ~~first nucleic acid molecule~~ is complementary or substantially complementary to a ribosome binding site (RBS) sequence.
257. **(Currently amended)** The system of claim 116, wherein ~~at least~~ a portion of the cis-repressive sequence element of at least 4 nucleotides in length ~~first nucleic acid molecule~~ is complementary or substantially complementary to a Kozak consensus sequence.
258. **(Currently amended)** The system of claim 254, wherein the ~~sequence of the~~ second stem-forming portion of the first nucleic acid molecule comprises all or part of an RBS sequence.
259. **(Currently amended)** The system of claim 254, wherein the ~~sequence of the~~ non-stem-forming portion of the first nucleic acid molecule comprises a YUNR sequence.

260. **(Previously presented)** The system of claim 254, wherein the non-stem-forming portion of the first nucleic acid molecule is 4, 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides in length.
261. **(Withdrawn)** The system of claim 254, wherein the non-stem-forming portion is between 13 and 50 nucleotides in length, inclusive.
262. **(Currently amended)** The system of claim 254, ~~whereby~~ wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is between 4 and 100 nucleotides, inclusive.
263. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is between 6 and 50 nucleotides, inclusive.
264. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is between 12 and 30 nucleotides, inclusive.
265. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is approximately 19 nucleotides.
266. **(Previously presented)** The system of claim 254, wherein the two stem-forming portions of the first nucleic acid molecule exhibit at least 66% complementarity.
267. **(Previously presented)** The system of claim 254, wherein the two stem-forming portions of the first nucleic acid molecule exhibit between 75 and 95% complementarity.
268. **(Previously presented)** The system of claim 254, wherein the two stem-forming portions of the first nucleic acid molecule exhibit approximately 85% complementarity.

269. **(Previously presented)** The system of claim 254, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least one area of non-complementarity.
270. **(Previously presented)** The system of claim 269, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least one bulge.
271. **(Previously presented)** The system of claim 254, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least two dispersed areas of non-complementarity.
272. **(Previously presented)** The system of claim 271, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least two dispersed bulges.
273. **(Previously presented)** The system of claim 254, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least three dispersed areas of non-complementarity.
274. **(Previously presented)** The system of claim 273, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least three dispersed bulges.
275. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule forms a single stable stem.
276. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation in the absence of a ligand.
277. **(Currently amended)** The system of claim 254, wherein the ~~first~~ second stem-forming portion of the first nucleic acid molecule comprises a ~~sequence complementary or substantially complementary to a sequence in the 5' portion of an~~ the ORF.

278. **(Previously presented)** The system of claim 254, wherein the first nucleic acid molecule comprises a start codon.
279. **(Previously presented)** The system of claim 278, wherein the first nucleic acid molecule comprises a spacer comprising one or more nucleotides between the 3' end of the second stem-forming portion and the start codon.
280. **(Withdrawn)** The system of claim 278, wherein all or part of the start codon is located within the second stem-forming portion.
281. **(Previously presented)** The system of claim 254, wherein the first nucleic acid molecule comprises one or more nucleotides at the 5' end that do not participate in the stem-loop structure.
282. **(Previously presented)** The system of claim 254, wherein the first nucleic acid molecule comprises between 5 and 50 nucleotides upstream of the 5' end of the first stem-forming portion.
283. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule comprises a ligand binding domain.
284. **(Currently amended)** The system of claim 254, wherein the first nucleic acid molecule comprises a third stem-forming portion of at least 4 nucleotides in length that is complementary or substantially complementary to the second stem-forming portion, wherein the first and third stem-forming portions form alternate stem-loop structures with the second stem-forming portion.
285. **(Currently amended)** The system of claim 284, wherein the ~~first and third~~ second stem-forming ~~portions~~ portion of the first nucleic acid molecule ~~comprise~~ comprises a portion ~~that is complementary or substantially complementary to~~ all or part of an RBS sequence.

286. **(Currently amended)** The system of claim 116, wherein the second nucleic acid molecule comprises a ~~portion comprising the sequence~~ YNAR sequence positioned 5' to the 5' ~~portion~~ end of the first stem-forming ~~sequence~~ portion.
287. **(Previously presented)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 6 and 50 nucleotides.
288. **(Previously presented)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 12 and 30 nucleotides.
289. **(Withdrawn)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is approximately 19 nucleotides.
290. **(Previously presented)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit at least 66% complementarity.
291. **(Previously presented)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit between 75 and 95% complementarity.
292. **(Withdrawn)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit approximately 85% complementarity.
293. **(Previously presented)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least one area of non-complementarity.
294. **(Previously presented)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least two dispersed areas of non-complementarity.

295. **(Withdrawn)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least three dispersed areas of non-complementarity.
296. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule comprises a nucleotide analog.
297. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule comprises a ligand binding domain.
298. **(Canceled)**
299. **(Withdrawn)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 and the second nucleic acid molecule has the sequence of taR10.
300. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 (SEQ ID NO:56) and the second nucleic acid molecule has the sequence of taR12 (SEQ ID NO:55).
301. **(Withdrawn)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 or a variant of crR10 that differs from crR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR10 or a variant of taR10 that differs from taR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.
302. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 (SEQ ID NO:56) or a variant of crR12 (SEQ ID NO:56) that differs from crR12 (SEQ ID NO:56) by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR12 (SEQ ID NO:55) or a variant of taR12 (SEQ ID NO:55) that differs from taR12 (SEQ ID NO:55) by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.

303. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule and the second nucleic acid molecule have an equilibrium association constant between 0.8×10^7 and 1.5×10^7 kcal/mol.